

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

n re Application of:

Mello et al.

Serial No.:

09/490,291

Group No.:

1653

Filed:

01/20/99

Examiner:

H. Schnizer

Entitled:

Novel Purification And Fiber Spinning Techniques For Protein Fibers

TRANSMITTAL OF FORMAL DRAWINGS

Official Draftsperson **BOX ISSUE FEE Assistant Commissioner for Patents** Washington, D.C. 20231

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In response to the NOTICE OF INFORMAL DRAWINGS or ALLOWABILITY, attached please find:

X 12 sheets of formal drawing(s) for this application.

Each sheet of drawing indicates the identifying indicia suggested in 37 CFR

§ 1.84(c) on the front side of the drawing.

Respectfully submitted:

Dated: APNIL 29, 2003

Vincent J. Ranucci, Esq.

Reg. No. 29,579

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Serial No.: 09/490,291

Filed: 01/202. Group Art Unit: 1653

Examiner: H. Schnizer Applicant: Mello et al.

Title: Novel Purification And Fiber

Spinning Techniques For Protein

Fibers

Atty. Docket No.: NA-1151

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FIG. 1

MRGSHHHHHHGSMASGRGGLGGQGAGAAAAAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAA AAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAAAAAAGGAGQGGYGGLGSQGTSGRGGLG GQGAGAAAAAAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAAAAAAAAAAAAAAGGAGQGGYGGLGSQ GTSGRGGLGGQGAGAAAAAAAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAAAAAAAAAGGAGQ GGYGGLGSQGTSGIRRPAAKLN

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ATGAGAGGATCGCATCACCCATCACGGATCCATGGCTAGCGGTAGAGGCGGGCTGGGTGGCCAG GGTGCAGGTGCGGCTGCCGCGGCAGCGGCCGCAGGCGGTGCCGGCCAAGGTGGCTATGGCGGC CTGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGGTGGCAGGTGCGGCTGCGGCTGCC GCGGCAGCGGCGCGGGCGAGGCGAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGT AGAGGCGGGCTGGCCAGGGTGCAGGTGCGGCTGCCGCGCGGCAGCGGCGCAGGCGGTGC CGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGG TGCAGGTGCGGCTGCCGCGCGCAGCGGCCGCAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCT GGGTTCTCAGGGGACTAGCGGTCCGGGCGGTTATGGTCCGGGTCAACAACTAGCGGTAGAGGCGGGCT GGGTGGCCAGGGTGCAGGTGCGGCTGCCGCCGCGCAGCGCCGCAGGCGGTGCCGGCCAAGGTG GCTATGGCGGCCTGGGTTCTCAGGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCGG CTGCGGCTGCCGCGGCAGCCGCAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGG GGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCCGCGCGCAGCGGCC GCAGGCGGTGCCGAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGGACTAGCGGTAGAGGCGGGCTG GGTGGCCAGGGTGCAGGTGCGGCTGCCGCCGCCAGCCGCCAGGCGGTGCCGGCCAAGGTGG CTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTCCGGGCGGTTATGGTCCGGGTCAACAACTAGCGG TAGAGGCGGGCTGGCCAGGGTGCAGGTGCGGCTGCCGCGCGCCAGCCGCCAGGCGGTG CCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGG GTGCAGGTGCGGCTGCCGCGGCAGCGGCCGCCAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCC TGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCCG CGGCAGCGGCCAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTA GAGGCGGGCTGGCCAGGGTGCAGGTGCGGCTGCCGCGCAGCGGCAGCGCAGGCGGTGCC GGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGGACTAGCGGTCCGGGCGGTTATGGTCCGGGTCAA CAAACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCCGCGGCAGCGGC CGCAGGCGGTGCCGAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGGACTAGCGGTAGAGGCGGGCT GGGTGGCCAGGGTGCGGCTGCGGCTGCCGCGCAGCGCCGCCAGGCGGTGCCGGCCAAGGTG GCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGG CTGCGGCTGCCGCGGCAGCCGCAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGG GGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCCGCGGCAGCGGCC GCAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTCCGGGCGGTTAT GGTCCGGGTCAACAACTAGTGGGATCCGTCGACCTGCAGCCAAGCTTAATTAG

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ŃRGSHHHHHHGSMASGRGGLGGQGAGAAAAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAA AAAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAAAAAGGAGQGGYGGLGSQGTSGRG GLGGQGAGAAAAAAAAGGAGQGCYGGLGSQGTSGPGGYGPGQQTSGRGGLGGQGAGAAAAAAA **AGGAĞQGGYGGLGSQGTSGRGG**LGGQGAGAAAAAAAAAGGAGQĞGYGGLGSQGTSGRGGLGGQGAG AAAAAAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAAAAAAGGAGQGGYGGLGSQGTSGP GGYGPGQQTSGRGGLGGQGAGAAAAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAA AAAGGAĞQGGYGGLGSQĞTSGRGGLGGQGAGAAAAAAAAAGGAĞQGGYGGLGSQĞTSGRGGLGGQG **AGAAAAAĀAAAAGGAGŌGGYGGLGSQGTSGPGGYGPGQQTSGRGGŪGGQGAGAAĀAAAAAAGGA**Ō QGGYGGLGSQGTSGRGĞLGGQGAGAAAAAAAAGGAĞQGGYGGLGSQĞTSGRGGLGGQGAGAAAA ÄAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAAAAAGGAGQGGYGGLGSQGTSGPGGYGP GOOTSGIRRPAAKLN

FIG. 4

ATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGCGGATCCATGGCTAGCGGTAGAGGCGGGCTGGGT GGCCAGGGTGCAGGTGCGGCTGCCGCGGCAGCGGCCAGGCGGTGCCGGCCAAGGTGGCTAT GGCGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCG GCTGCCGCGGCAGCGGCGGGCGGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACT AGCGGTAGAGGCGGGCTGGCCAGGGTGCAGGTGCGGCTGCCGCGCCGCCAGCGCCAGG CGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGGACTAGCGGTAGAGGCGGGCTGGGTGG CCAGGGTGCAGGTGCGGCTGCCGCGCGCAGCGGCGAGGCGGTGCCGGCCAAGGTGGCTATGG AGGTGGCTATGGCGGCCTGGGTTCTCAGGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGG TGCGGCTGCGGCTGCCGCCAGCGCCGCCGCCAGGCGGCCAAGGTGGCTATGGCGGCCTGGGTTC TCAGGGGACTAGCGGTAGAGGCGGGCTGGCCAGGGTGCAGGTGCGGCTGCCGCGGCAG CGGCCGCAGGCGGTGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGCG GGCTGGGTGGCCAGGTGCGGCTGCCGCCGCGCAGCCGCCAGGCGGTGCCGGCCAA GGTGGCTATGGCGGCCTGGGTTCTCAGGGGGACTAGCGGTCCGGGCGGTTATGGTCCGGGTCAACAAACT AGCGGTAGAGGCGGGCTGGCCAGGGTGCAGGTGCGGCTGCCGCCGCCGCCAGGCAGCGCCAGG CGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGGACTAGCGGTAGAGGCGGGCTGGGTGG ${\tt TGCCGCGGCAGCGGCCGGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAG$ CGGTAGAGGCGGGCTGGCCAGGGTGCAGGTGCGGCTGCCGCGCAGCGGCCGCAGGCG GTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTCCGGGCGGTTATGGTCCGG GTCAACAAACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCCGCGGCA GCGGCCGCAGGCGTGCCGAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGC AGGTGGCTATGGCGGCCTGGGTTCTCAGGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGG TGCGGCTGCGGCTGCCGCAGCGCCGCAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTC TCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCCGCGGCAG CGGCCGCAGGCGGTGCCGAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTCCGGGCG GTTATGGTCCGGGTCAACAACTAGTGGGATCCGAATTCGAGCTCCGTCGACAAGCTTCGAGCACCACC ACCACCACCACTGA

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FIG. 6

ATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGGATCCGAATTCGTGGATATGGAGGTCTTGGTGGA CAAGGTGCCGGACAAGGAGCTGGTGCAGCCGCCGCAGCAGCAGCTGGTGGTGCCGGACAAGGAGGATA TGGAGGTCTTGGAAGCCAAGGTGCTGGACGAGGTGGACAAGGTGCAGCCGCAGCCGCAGCTG GAGGTGCTGGTCAAGGAGGATACGGAGGTCTTGGAAGCCAAGGTGCTGGACGAGGAGGATTAGGTGGA CAAGGTGCAGGTGCAGCAGCAGCTGGAGGTGTCGGACAAGGAGGACTAGGTGGACAAGGTGCTGG ACAAGGAGCTGGAGCAGCTGCTGGTGGTGCCGGACAAGGAGGATATGGAGGTCTCGGAA GCCAAGGTGCAGGACGAGGTGGATCAGGTGGACAAGGGGGCAGCAGCAGCAGCAGCAGCTGGAGGT GCCGGACAAGGAGATATGGAGGTCTTGGAAGCCAAGGTGCAGGACGAGGTGGATTAGGTGGACAGGG TGCAGGTGCAGCAGCAGCAGCCGGAGGTGCTGGACAAGGAGGATACGGTGGTCTTGGTGGAC AAGGTGCCGGACAAGGTGGCTATGGAGGACTTGGAAGCCAAGGTGCTGGACGAGGAGGATTAGGTGGA CAAGGTGCAGGTGCAGCAGCAGCTGGAGGTGCCGGACAAGGAGGACTAGGTGGACAAGGAGCTGG AGCAGCCGCTGCAGCAGCTGGTGCCGGACAAGGAGGATATGGAGGTCTTGGAAGCCAAGGTGCTG GACGAGGTGGACAAGGTGCAGGCGCAGCAGCAGCCGGAGGTGCTGGACAAGGAGGATACGGT GGACAAGGTGCCGGACAAGGAGGCTATGGAGGACTTGGAAGCCAAGGTGCTGGACGAGGAGGATTAGG TGGACAAGGTGCAGCAGCAGCAGCAGCAGCAGCTGCAGGTGCCGGACAAGGAGGATTAGGTG GACAAGGTGCAGCAGCAGCAGCAGCTGGAGGTGCTGGACAAGGAGGATTAGGTGGACAAGGT GCTGGACAAGGAGCTGGAGCAGCCGCTGCAGCAGCAGCTGGTGGTGTTAGACAAGGAGG CCGGAGGTGCTGGACAAGGAGATATGGTGGTCTTGGTGGACAAGGTGTTGGACGAGGTGGATTAGGTG GACAAGGTGCAGCGCAGCGGCAGCTGTTGGTGCTGGACAAGGAGGATATGGTGGTGTTCTGGGG CGTCTGCTGCCTCTGCAGCTGCATCCCGTTTGTCTTCTCCTCAAGCTAGTTCAAGAGTTTCATCAGCTGTT TCCAACTTGGTTGCAAGTGGTCCTACTAATTCTGCGGCCTTGTCAAGTACAATCAGTAATGTGGTTTCAC AAATAGGCGCCAGCAATCCTGGTCTTTCTGGATGTGATGTCCTCATTCAAGCTCTTCTCGAGCACCACCA CCACCACCACTGAA

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AGGAGQGGYGGLGSQGAGRGGLGĞQGAĞAAAAAGGVGQGGLGĞQGAGQAGAAAAAAGĞAGQGGYG GLGSQGAGRGGSGGGGAGAAAAAAGGAGQGGYGGLGSQGAGGAGGAGAAAAAAAAGGAGQGGYG GLGGQGAGQGGYGGLGSQGAGRGGLGGQGAGAAAAAGGAGQGGLGGQGAGAAAAAAGGAGQGGYGGL GSQGÄGRGĞQGAGAAAAÄAGGAGQGGYĞGQGAGQGGYGGLĞSQGAGRGGLGGQGAGAAAAÄAAAGGA GQĞGLGGQGĀGAAAAAAGGAGQGĞLGGQGĀGQGĀGAAAAAAAĀĀAAGGVRQGGŸGGLGSQGAGRGGQ GAGAAAAAAGGAGQGGYGGLGGQGVGAĞGLGĞQGAGAAAVGAGQGGYGĞVGSGASAAŠAAASRLŠS PQASSRVSSAVSNLVASGPTNSAALSSTISNVVSQIGASNPGLSGCDVLIQALLGHHHHHH

FIG. 8

AEIYNKDGNKVDLYGKAVGLHYFSKGNGENSYGGNGDMTYARLGFKGETQINSDLTGYGQWEY NFQGNNSEGADAQTGNKTRLAFAGLKYADVGSFDYGRNYGVVYDALGYTDMLPEFGGDTÄYSD DFFVGRVGGVATŸRNSNFFGLVDGLNFAVQYLGKNERDTARRSNGDGVGGSISYEYEGFGIVGAY GAADRINLQEAQPLGNGKKAEQWATGLKYDANNIYLAANYGETRNATPITNKFTNTSGFANKTQ DVLLVAQYQFDFGLRPSIAYTKSKAKDVEGIGDVDLVNYFEVGATYYFNKNMSTYVDYIINOIDS DNKLGVGSDDTVAVGIVYOFA

FIG. 9

ATGAGAGGATCGCATCACCATCACGGATCCATGGCTAGCGGTGACCTGAAAAACAA AGTGGCCCAGCTGAAAAGGAAAGTTAGATCTCTGAAAGATAAAGCGGCTGAACTGAAACAAG AAGTCTCGAGACTGGAAAATGAAATCGAAGACCTGAAAGCCAAAATTGGTGACCTGAATAAC ACTAGTGGGATCCGTCGACCTGCAGCCAAGCTTAATTAG

FIG. 10

MRGSHHHHHHGSMASGDLKNKVAQLKRKVRSLKDKAAELKQEVSRLENEIEDLKAKIGDLNNTSGIRRPAA KLN

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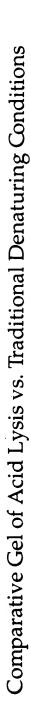
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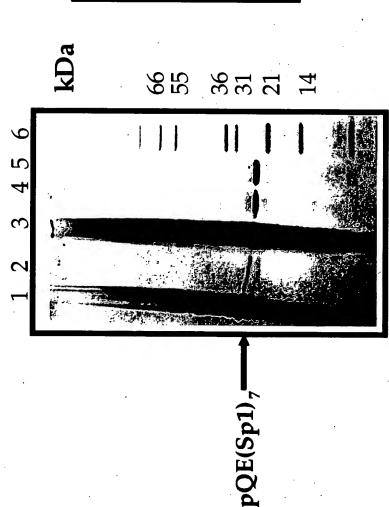
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Molecular weight markers Propionic acid lysate 6M guanidine lysate, Ni-NTA purified Formic acid lysate Ni-NIA purified 8M urea lysate Formic Lysate, Ŋ. 9



Gel of QAE-Sephadex Purification of Propionic Acid (PA) ExtractedpET[(Sp1)₄/(Sp2)₁]₄Protein

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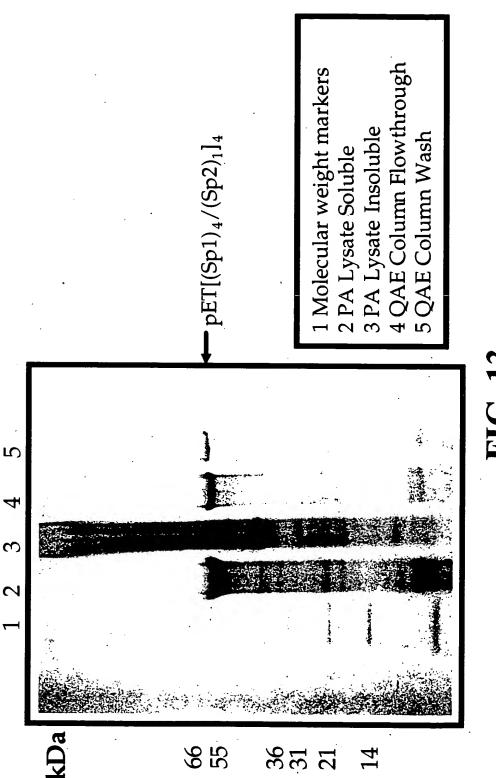


FIG. 1

Fibers Sheet 8 of 12 Atty. Docket No.: NA-1151 1 Propionic Acid/Gdn-HCl Lysate (precolumn) QAE Column Flowthrough Molecular weight markers and Guanidine-HCl Extracted pET[(SP1)₄/(SP2)₁]₄ Protein QAE Column Wash QAE-Sephadex Purification of Propionic Acid 0 m kDa 99 36 55 **FIG. 14** S. Salval 3 2 $pET[(SP1)_4/(SP2)_1]_4$

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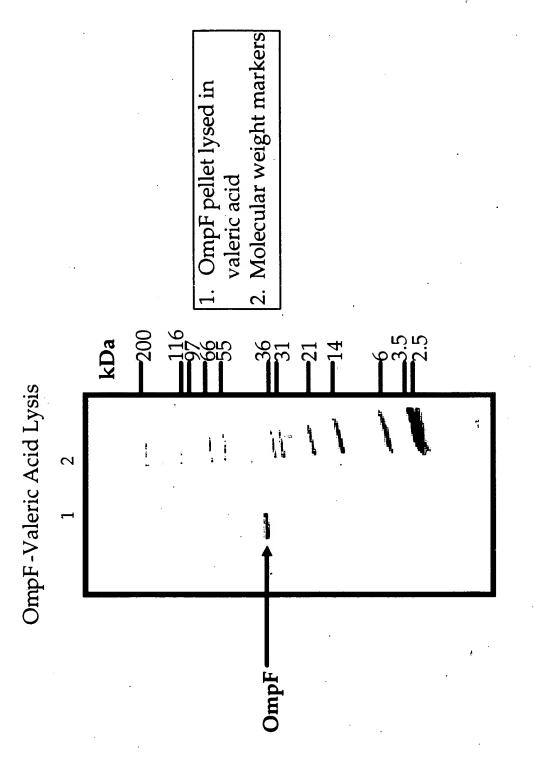


FIG. 15



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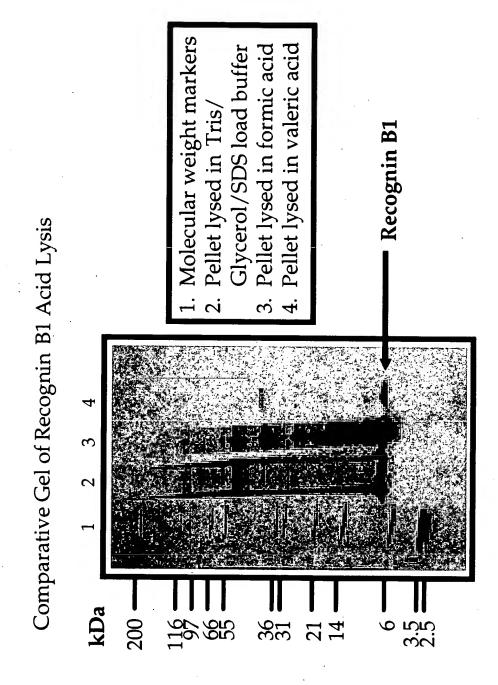


FIG. 16



pETNcDS fiber under light microscopy. Spun from 25%

protein solution into 90% methanol coagulation bath.

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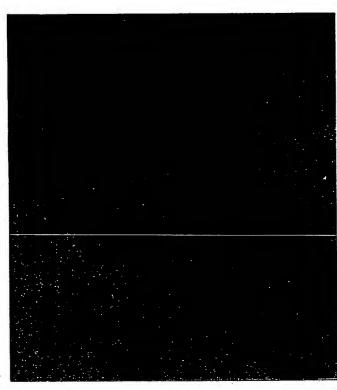
itle: Novel Purification And Fiber

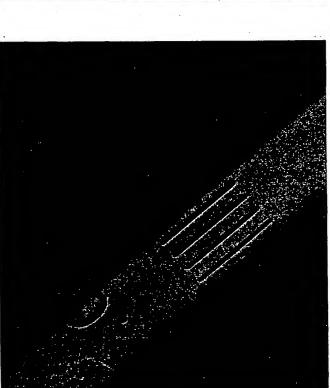
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Polarized light w/tint plate



 $pQE[(SP1)_4/(SP2)_1]_4$ fiber under light microscopy. Spun from a 12.5% protein solution into 90% methanol coagulation bath.

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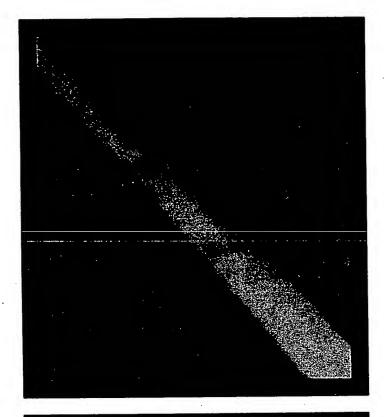
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White light

Polarized light w/ tint plate